1107-Pos Board B17
Non-Esterified Fatty Acids (NEFAs) Generate Distinct Aβ42 Oligomers via Two Distinct Aggregation Pathways
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In Alzheimer's disease (AD), soluble oligomers of amyloid-β (Aβ) are believed to be primary neurotoxic species responsible for early synaptic dysfunction and cognitive decline. The rate of Aβ aggregation is known to be significantly affected in the presence of anionic interfaces such as lipid, fatty acids & other surfactants. Here, we present the effect of saturated non-esterified fatty acids (NEFAs) on the rate of Aβ aggregation. We have observed that NEFAs induce more than one pathway of Aβ aggregation which is dictated by both ratio of Aβ42 : NEFAs as well as NEFAs respective critical micelle concentrations (CMC). More importantly, we observed that irrespective of their carbon chain lengths, NEFAs generate primarily two types of low molecular weight oligomeric species ; a) near CMC concentration, NEFAs increased the rates of Aβ aggregation towards fibril formation that generated 12-18mers, and b) at concentration above CMC, NEFAs failed to show any aggregation and generated 4-5mers, while oligomeric 12-18mers seems to adopt 'on pathway' towards fibril formation, the 4-5mers formed via an alternate pathway called 'off-pathway' that did not form fibrils. These oligomers generated were characterized using biophysical techniques like thioflavin-T (ThT) fluorescence, immunoblotting, atomic force microscopy (AFM) and circular dichroism (CD). The results from these analyses also showed that these oligomers are generated via two different pathways. All these data are presented and discussed.

1108-Pos Board B18
Topology of His Residues in Amyloid β Protein Fibrils
Hiroaki Komatsu, Paul H. Axelsen.
Oxidatively damaged lipid membranes are known to promote the aggregation of amyloid β (Aβ) proteins into fibrils. When lipid membranes contain ω-6 polyunsaturated fatty acyl chains and subjected to oxidative stress, 4-hydroxy-2-nonenal (HNE), a highly reactive short chain alkenal, is typically produced. We previously demonstrated that HNE modifies the three His residues of Aβ proteins by Michael addition, which increases their affinity for the lipid membrane surface, and promotes the aggregation of unmodified Aβ proteins into fibrils. In this report, HNE-promoted Aβ fibrils were studied by negative-staining electron microscopy and shown to have morphologies identical to fibrils formed without HNE. The binding of antibodies specific for HNE-His adducts was studied by colloidal gold immunoelectron microscopy. Results indicate that the His13 residue of Aβ protein (the 42 residue form) was inaccessible, while the His14 residue was accessible. These results agree with some of the published molecular structure models of Aβ protein fibrils.

Membrane Protein Structure I

1109-Pos Board B19
Analyzing Inter-Residue Interactions for Structure Modeling of Helical Membrane Proteins
Zhijun Li, Jun Gao, Vagmita Pabuwal.
Transmembrane (TM) proteins are estimated to account for ~20-30% of the human genome and serve as important drug targets. Despite the significant progress in experimental techniques, TM protein structure determination remains a challenge in general. Computational approaches, including de novo and comparative structure predictions, have played a significant role in structural and functional studies of membrane proteins, as well as in their structure-based drug design efforts. A major challenge of membrane protein structure prediction is to assemble individual helices into high-quality tertiary structures. Analyzing inter-residue interactions within membrane proteins is of significance to developing novel tools to tackle this challenge. In this work, we first identified four approaches for determining inter-residue interactions in protein structures and studied their correlation to individual quality measures. It was found that the best correlation was achieved by the approach focusing exclusively on favorable inter-residue interactions. Next, this best approach was applied to the analysis of multiple datasets of helical membrane protein structure models, including a dataset of homologous structure pairs with the sequence identity at the twilight zone. A simple measure was elucidated that directly correlates with the quality of these structure models. These results suggest a useful tool for computational modeling of membrane protein structures. This work was supported by the grant (1R15GM084404) from National Institute of Health.

1110-Pos Board B20
Protein Folding and Membrane Insertion via the Protein Translocon
James C. Gumbart, Christophe Chipot, Klaus Schulten.
Nascent membrane proteins typically insert in a sequential fashion into the membrane via a protein-conducting channel, the Sec translocon. How transmembrane (TM) segments are recognized or what secondary structure they possess inside the channel remains largely unknown. Experiments and simulations measuring the free energy of insertion for various amino acids are in disagreement, with the former finding a much narrower range than the latter. We suggest that this disagreement is resolved by assuming a two-stage insertion process wherein the first step, the insertion into the translocon, is energized by protein synthesis and, therefore, has an effectively zero free-energy cost; the second step, the insertion into the membrane, invokes the translocon as an intermediary between the fully hydrated and the fully inserted locations. Using free-energy perturbation calculations, the effective transfer free energies from the translocon to the membrane have been determined for both arginine and leucine amino acids carried by a helical poly-leucine segment, which when compared to transfer from water, result in the same compression as observed in the experiment-based scale. We also show, using 2D potential-of-mean-force calculations, that secondary structure formation inside the translocon is similar to that in water with an additional entropic contribution from the narrowing of the channel, validating the assumption of helicity for the background poly-leucine segment.

1111-Pos Board B21
A general and Efficient All-Atom Simulation Method to Determine the Equilibrium Orientation of Transmembrane Proteins in Membranes
Kethika Kulleperuma, John Holyoake, Régis Pomès.
Knowledge of the spatial orientation of transmembrane (TM) proteins is broadly useful in both in-vitro and in-silico approaches that aim to elucidate the dynamic and functional properties of TM proteins. Such approaches include site-directed spin labeling, cys-scanning mutagenesis, and atomistic molecular dynamics (MD) simulations using lipid bilayers. Because three-dimensional structures of TM proteins are generally obtained in a detergent solvent, these structures do not reveal the native orientation of the protein in the lipid membrane. For this reason, we aimed to develop a general and computationally-efficient MD approach to predict the most favorable orientation of TM proteins in a lipid membrane. In order to avoid the long relaxation time scales characterizing protein and lipid bilayer dynamics, our method treats the TM protein as a rigid body in a membrane-mimetic hydrated octane slab, allowing the protein to reach a stable orientation within 10 ns. The method was systematically tested on alpha-helical and beta-barrel TM proteins, each with different starting orientations in the hydrated octane slab. Each protein attained a consistent orientation irrespective of its starting orientation. Furthermore, the converged orientations are in good agreement with the known orientations of these test proteins in lipid bilayers. These results indicate that this method is reliable as a general protocol that can be used to determine the orientation of TM proteins of known structure.

1112-Pos Board B22
Insertion Properties of Cftr Explored with High Throughput, Coarse Grain Molecular Dynamics
Benjamin A. Hall, Alan Chetwynd, Mark S.P. Sansom.
Transmembrane helix insertion into the membrane occurs through a complex process, involving dedicated cellular machinery. Recent experimental work has been able to show that the insertion of peptides by the translocon shows high correlation with hydrophobic scales based on water/octanol partitioning, but that the absolute energies of insertion of different amino acids are consistently different by an order of magnitude. Similarly, energies of transmembrane insertion from explicit energy calculations on detailed molecular model also appear to differ, by up to 2 orders of magnitude. Coarse grain (CG) techniques are an increasingly popular approach for the molecular modelling of biomolecules, which increase the effective timescale or system size which can be modelled compared to more common atomistic techniques. We adopt a high throughput, CG approach to understanding helix insertion into the membrane. Using self assembly of systems of peptides derived from the cystic fibrosis protein, we are able to predict transmembrane insertion energies with a correlation coefficient of up to 0.86, and energies within a factor of 2 of the experimentally determined energies. Additionally, we show that the insertion behaviour observed is sensitive to membrane thickness, and in agreement with explicit energy calculations and experimental evidence, find that thinner membrane bilayers favour a transmembrane conformation. Alongside results from PMF calculations, the results here appear to suggest that the energy